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Donkey milk: chemical make-up, biochemical features, nutritional worth, and possible human health benefits - Current state of scientific knowledge

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ABSTRACT

Milk and milk derivatives are widely consumed because of their high nutritional density. Donkey milk and milk products have been consumed since ancient times. The use of donkey milk in the human diet is gaining popularity. The abundance of antibacterial components and protective elements in donkey milk sets it apart from the milk of other animals. Like human milk, donkey milk has low fat, high lactose, and low casein/whey protein ratio. Donkey milk whey protein's anti-proliferative properties imply lung cancer treatment. Alpha-lactalbumin, a type of protein, has been found to have antiviral, anticancer, and anti-stress properties. Donkey milk, like human milk, includes a low amount of casein and a smaller quantity of beta-lactoglobulin than cow milk. Donkey milk is an alternative for newborns with cow milk protein allergy and lactose intolerance since it has a higher amount of lactose, improves palatability, and prevents allergies. Osteogenesis, arteriosclerosis therapy, cardiac rehabilitation, accelerated aging, and hypocholesterolemic diets are some areas where donkey milk is beneficial. Since it contains probiotic

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lactobacilli strains, fermented beverages can be made with donkey milk. Donkey milk moisturizes skin due to its high vitamin, mineral, and polyunsaturated fatty acid content. The chemical makeup and potential therapeutic benefits of donkey milk warrant additional research. This has led to a rise in interest in producing dairy goods derived from donkey milk. Donkey milk has been used to make cheese, ice cream, milk powder, and even some experimental useful fermented drinks. The present article summarises what we know about donkey milk's chemical makeup, biological functions, nutritional worth, and possible human health benefits.

1 Introduction

Colostrum and transitional milk in each mammal provide passive immunization and sustenance. Human breast milk is the best nutrition for infants in the first four months; however, in some cases (lack of milk ejection, orphans, or sick mothers), a healthy substitute is needed (Cimmino et al. 2023). Due to the high demand for milk and other dairy products among human populations, cows have become the principal dairy animal species worldwide. However, cow milk is inappropriate for 5–15% of newborns with cow milk protein allergies (Aspri et al. 2017; Martini et al. 2021; Lejaniya et al. 2021a; Lejaniya et al. 2021b; Garhwal et al. 2022). Clinical cross-reactivity across ruminant milk has sometimes shown extremely high sensitivity in these people. Milk from domesticated mammals other than cows have recently become commercially valuable due to their high nutritional value and potential medical applications (Chandran et al. 2021; Patange et al. 2022a; Patange et al. 2022b; Baloš et al. 2023; Krishnan et al. 2023). More and more studies have been documented about donkey milk in recent years, confirming its growing interest due to the hypoallergenic features and other unusual and health-promoting aspects noted by numerous authors.

Donkey milk (ass milk/jenny milk) is made by milking a healthy domesticated donkey (*Equus asinus*). North Africa's Nile Valley domesticated donkeys approximately 6000 B.C. They swiftly spread across India, Asia, South Europe and South America. Food and Agriculture Organization identifies three varieties of donkeys native to India: the Indian, the Indian wild donkey, and the Kiang. Rann of Kutch (Gujarat) is home to one of the two main types of Indian wild donkeys, whereas Kiang's can be found in neighbouring regions like Sikkim and Ladakh (Pilla et al. 2010; Aspri et al. 2017; Cimmino et al. 2023). Figure 1 shows the distribution of donkey milk production worldwide and the nations that produce the most of it.

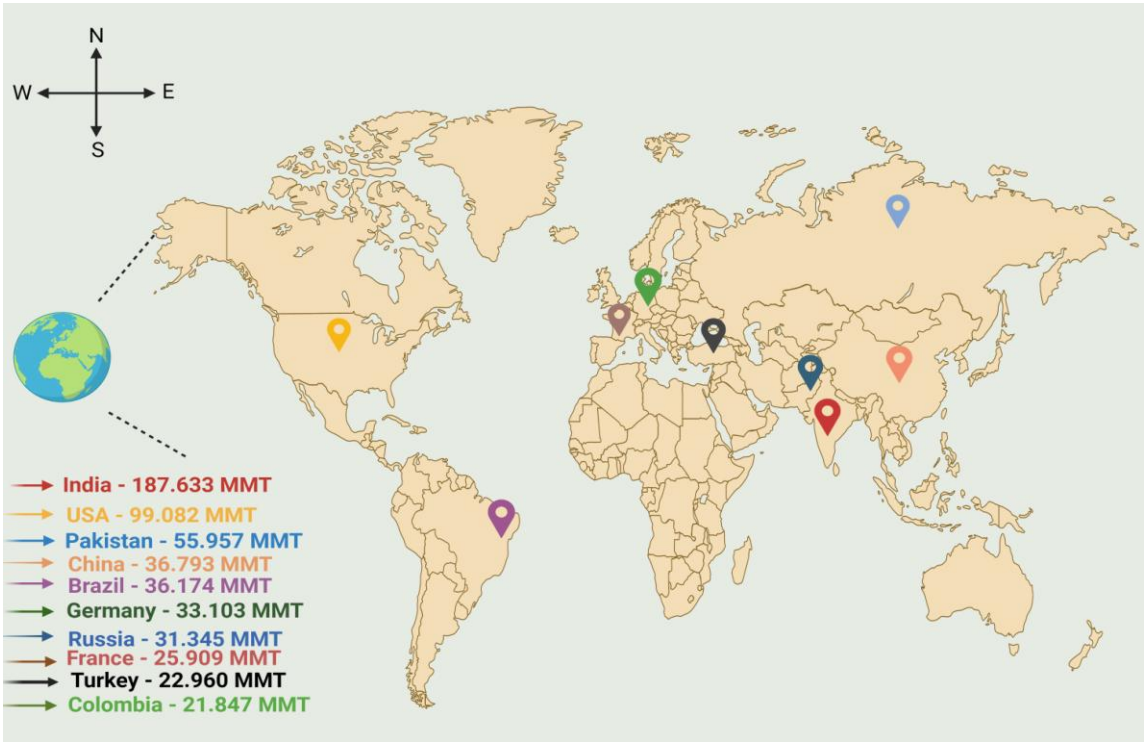

Figure 1 Distribution of donkey milk production around the world and the nations that produce the most of it
(Designed with Biorender premium software; https://app.biorender.com/)

Donkey milk has been used for centuries as a remedy for various ailments, including asthma, joint pain, gastritis, and bronchitis. It is commercially accessible and can help infants, those with cow's milk protein allergies, and older people (Martini et al. 2018a; Karami and Akbari-adergani 2019). Donkey milk's health benefits have piqued the public's attention. Donkey milk is a "pharma food" due to its nutritional, nutraceutical, and functional properties. Due to its biochemistry, like human milk, donkey milk can be used as a safe and nutritious substitute for children with cow milk allergies (Mottola et al. 2018; Garhwal et al. 2022). As well as sharing nutritional similarities with human milk, this alternative possesses similar hypo-allergenicity, immunological homeostasis conditions, and antibacterial action. Donkey's milk may be an option for a bovine milk protein-allergic baby if breast milk is unavailable (Vincenzetti et al. 2017; Conte and Panebianco 2019). Infants love and tolerate donkey milk because it tastes like human milk. Crohn's and ulcerative colitis patients can be benefited from this compound's hypo-allergic, anti-aging and antimicrobial characteristics (Martini et al. 2021; Baloš et al. 2023; Cimmino et al. 2023). Although all components of donkey milk have nutritional and physiological effects, the proportion of whey protein is considered significant (Derdak et al. 2020). This review article summarizes donkey milk's nutritional characteristics and potential health advantages.

2 Physico-chemical composition of donkey milk

Donkey milk tastes, smells, and looks like water. Neither the number of somatic cells nor total microorganisms in milk is very high (Massouras et al. 2020). Protein content varies significantly from species to species and is affected by factors like breed, lactation stage, diet, climate, parity, season, and udder health, as reported by the vast majority of authors (Garhwal et al. 2022; Saleena et al. 2022a; Saleena et al. 2022b). There is promising evidence that protein in milk can bridge the gap between nutrition, dietetics, and therapy (Vincenzetti et al. 2017). Immunoglobulins, lactoferrin, lactoperoxidase, and lysozyme are milk-derived antibacterial proteins. To function, immunoglobulins (secretory Ig-A, Ig-G and Ig-M) rely on a mechanism involving antigen-antibody interactions (Aspri et al. 2017; Chandran and Radhakrishnan 2019; Papademas et al. 2022). Differences in nutrient content between donkey milk and the milk of other mammals, including humans, are presented in Table 1.

2.1 Milk proteins

Milk contains a variety of proteins, including milk fat globule proteins, caseins, whey proteins, enzymes, and others (Brumini et al. 2016; Saleena et al. 2022a; Saleena et al. 2022b). Donkey milk is far lower in protein than cow milk (1.5-1.8 g/100g) and more like human and mare milk (Vincenzetti et al. 2017; Vincenzetti et al. 2021). Donkey milk is a nutritious alternative for cow's milk-allergic newborns. Milk lipids are nutritionally valuable in immune inflammatory response control because of their significant parallels to human milk (Mottola et al. 2018; Bhardwaj et al. 2020). Whey proteins in donkey milk are primarily alpha-lactalbumin, beta-lactoglobulin, and lysozyme. Alpha-lactalbumin in donkey milk has two isoelectric points (Kumar et al. 2021; Papademas et al. 2022). Antiviral, anticancer, and stress-relieving effects have been discovered in

Table 1 Differences in nutrient content between donkey milk and the milk of other mammals, including humans

Nutrients	Donkey milk	Camel milk	Cow milk	Goat Milk	Sheep milk	Human milk
Fat (g/100mL)	0.3-1.8	4.5	3.5	3.5	6.1	3.4
Protein (g/100mL)	1.3-1.8	3.5	3.4	3.3	6.21	1.1
Lactose (g/100mL)	5.8-7.4	4.4	4.5	4.1	4.8	6.5
Minerals (g/100mL)	0.3-0.5	0.7	0.7	0.86	5.5	0.21
Solids-not-fat (g/100mL)	9.018	8.6	9.1	8.75	10.33	8.9
Total solids (g/100mL)	8.8-11.7	16.89	13.12	13.2	18.75	12.75
Cholesterol (mg/100g)	8.6	34.5	5	11	27	14
Calcium (g/100mL)	6.89	1.43	1.20	1.34	2.00	3.20
Phosphorus (g/100mL)	1.596	1.16	1.3	1.08	0.15	0.13
Saturated fatty acids (g/100mL)	67.6	51.9	67.73	70.42	65.17	46.60
Monounsaturated fatty acids (g/100mL)	15.80	39.60	27.3	25.67	24.29	43.55
Polyunsaturated fatty acids (g/100mL)	16.60	8.46	5.25	4.08	2.45	9.85
Water (%)	92.5	87-90	87	82.46-89.05	80.62	87.5

Source: Madhusudan et al. (2017); Souroullas et al. (2018); Bhardwaj et al. (2020); Prasad (2020); Kumari and Sharma (2021); Bertino et al. (2022); Garhwal et al. (2022); Papademas et al. (2022); Baloš et al. (2023)

alpha-lactalbumin (Aspri et al. 2017; Cimmino et al. 2023). It has also been demonstrated that alpha-lactalbumin has an anti-inflammatory effect by suppressing cyclooxygenase-2 and phospholipase A2 (Papademas et al. 2022). Beta-lactoglobulin, abundant in cow milk but missing in human milk, is often allergenic to children. Donkey milk has a beta-lactoglobulin level similar to mare milk (approximately 40% of whey proteins) but lower than cow milk. Donkey milk is hypoallergenic (Brumini et al. 2016; Conte and Panebianco 2019).

Donkey milk is the best alternative to human milk for children with cow milk protein allergy due to its palatability, nutritional adequacy, clinical tolerability, and physiological functions like digestive activity molecules, antibacterial substances, growth factors, and hormones (Bhardwaj et al. 2020; Garhwal et al. 2022). Caseins in donkey milk differ from those in cow milk in that alpha-S-1 casein and beta-casein, in their various phosphorylated and glycosylated forms, are present, whereas kappa-casein and alpha-S2-casein are present only in trace levels (Vincenzetti et al. 2017; Kumar et al. 2021; Cimmino et al. 2023).

The amounts of allergenic components in milk may affect tolerance. Beta-lactoglobulin in donkey milk comes in three different genetic forms; one contains three amino acid replacements, while the other forms each have two amino acid exchanges (Mottola et al. 2018; Baloš et al. 2023). In contrast to ruminant milk, which contains beta-lactoglobulin as a dimer, donkey milk only contains a single copy of this protein. As a member of the lipocalin family, beta-strong lactoglobulin's affinity for a wide variety of chemicals has led to many hypotheses regarding the protein's role in the body (Martini et al. 2021). Beta-lactoglobulin forms complexes with folic acid, suggesting that functional meals could use these complexes to transport folic acid. Hydrophobic ligand transport and absorption, enzyme regulation, and newborn latent immunological response are all linked to this protein (Bhardwaj et al. 2020; Cimmino et al. 2023). Donkey milk, similar to human milk but contains a higher concentration of amino acids like Ser, Glu, Arg, and Val and a lower percentage of Cys, could be a novel dietetic meal and breast milk substitute (Martini et al. 2021; Garhwal et al. 2022).

2.2 Milk fat

Donkey milk's fat content is lower than that of human milk, ranging from 0.03 to 1.18 kg/kg in a nonlinear fashion from partum to the end of lactation, making it a viable option for use in diet treatment to decrease the incidence of cardiovascular disease, autoimmune disease, and inflammatory diseases (Bhardwaj et al. 2020). The human diet's 52.2% polyunsaturated fatty acids (PUFA), low omega-6 to omega-3 ratio, and low atherogenic and thrombogenic indices are advantageous (Garhwal et al. 2022). To reduce blood cholesterol levels, halting the development of atherosclerotic plaques and eliminating the danger of high blood pressure, coronary heart disease and blood clots (Martini et al. 2021). Donkey milk is a welcome addition to the human diet because of the significant risk of fatty acid deficiency, especially PUFA omega-3 fatty acids required for optimal growth, cognitive development, and cardiovascular health (Ragona et al. 2016). Donkey milk contains 9.0 g/100 g and 5.1 g/100 g of total fatty acids, respectively, of the PUFAs linoleic acid (C18:2) and linolenic acid (C18:3) (Bhardwaj et al. 2020). Donkey milk fat globules are significantly smaller than those found in bovine milk, with an average diameter of 1.92 microns (Baloš et al. 2023). Bovine milk fat globules are roughly twice as large as donkey milk fat globules in average diameter. Donkey milk may be easier to digest because its fat globules are smaller than other kinds of milk, providing more surface area for lipase action (Pilla et al. 2010; Garhwal et al. 2022).

2.3 Lactose

Donkey milk contains more lactose (between 5.8 and 7.4%) than cow milk or human milk. The large amount is beneficial because it makes breast milk more appealing to infants and helps them absorb calcium, which is essential for the growth of healthy bones (Bhardwaj et al. 2020). Donkey milk has a pleasant flavour because of the lactose in it, and it is a valuable source of galactose, which is required for proper nervous system growth. Inadequate for 10 to 60% of the lactose intolerant population due to donkey milk's high lactose content from ruminant milk (Aspri et al. 2017; Cimmino et al. 2023). Those who have trouble digesting lactose may be able to eat fermented milk products like yoghurt because they include living bacteria that break down the lactose into lactic acid. Due to triacylglycerols with high unsaturation degrees and minor components with low partition number values, donkey milk has a higher PUFA concentration (Conte and Panebianco 2019).

2.4 Minerals

The growth and development of a human skeleton rely heavily on mineral intake. Milk is a primary food source of minerals. Depending on the breed and the stage of lactation, the typical composition of donkey milk ranges from 0.3 to 0.9 g/100 g (Vincenzetti et al. 2021). This exceeds cow milk but falls short of human milk. Donkey milk is rich in calcium, iron, sodium, potassium, phosphorus, magnesium, zinc, and copper (Papademas et al. 2022).

2.5 Vitamins

It is well established that mare milk has substantially more vitamin C than cow milk, and studies on the vitamin content of

donkey milk are only being started. Human milk has more significant quantities of niacin, whereas donkey milk has higher concentrations of cyanocobalamin, thiamine, riboflavin and vitamin C (Pilla et al. 2010; Vincenzetti et al. 2021; Papademas et al. 2022).

2.6 Enzymes

Different from the milk of other mammals, donkey milk contains enzymes with unique properties, such as a bactericidal effect. Donkey milk has a solid microbial inhibitory activity compared to other mammalian milk. Because of the large concentrations of lysozyme and lactoferrin in donkey milk, this is thought to be the case (Mottola et al. 2018; Papademas et al. 2022). Donkey milk has a low bactericidal concentration (0.02 g/L) due to its high lysozyme concentration (1.0 g/L). There may be as much as 4,000 mg/L of lysozyme in a single serving of donkey milk (Garhwal et al. 2022). There is around twice as much lactoferrin in donkey's milk as in bovine milk. Donkey milk only had 10^4 CFU/mL of bacteria and other microbes (Martini et al. 2021; Vincenzetti et al. 2021). Figure 2 shows donkey milk's physicochemical components.

3 Shelf life of donkey milk

Milk shelf life is crucial as it helps in its storage, processing, packaging and supply. It is the actual time in which it deteriorates to an unacceptable level. The higher the shelf life, the better its acceptability would be. Mare and donkey milk samples collected aseptically were incubated at 37 °C for 24 h, and periodical change in acidity and pH in equine milk was regularly studied at 2 h intervals (Bhardwaj et al. 2020). Shelf-life results indicated that mare and donkey milk was stable at 37 °C up to 8 h and 10 h, respectively. The presence of lysozyme, a natural preservative, extends the storage life of raw donkey's milk (Vincenzetti et al. 2021; Cimmino et al. 2023).

4 Human health benefits of donkey milk

Due to its high nutritious content and positive effects, as depicted in Figure 3, donkey milk is often consumed. In recent years, it

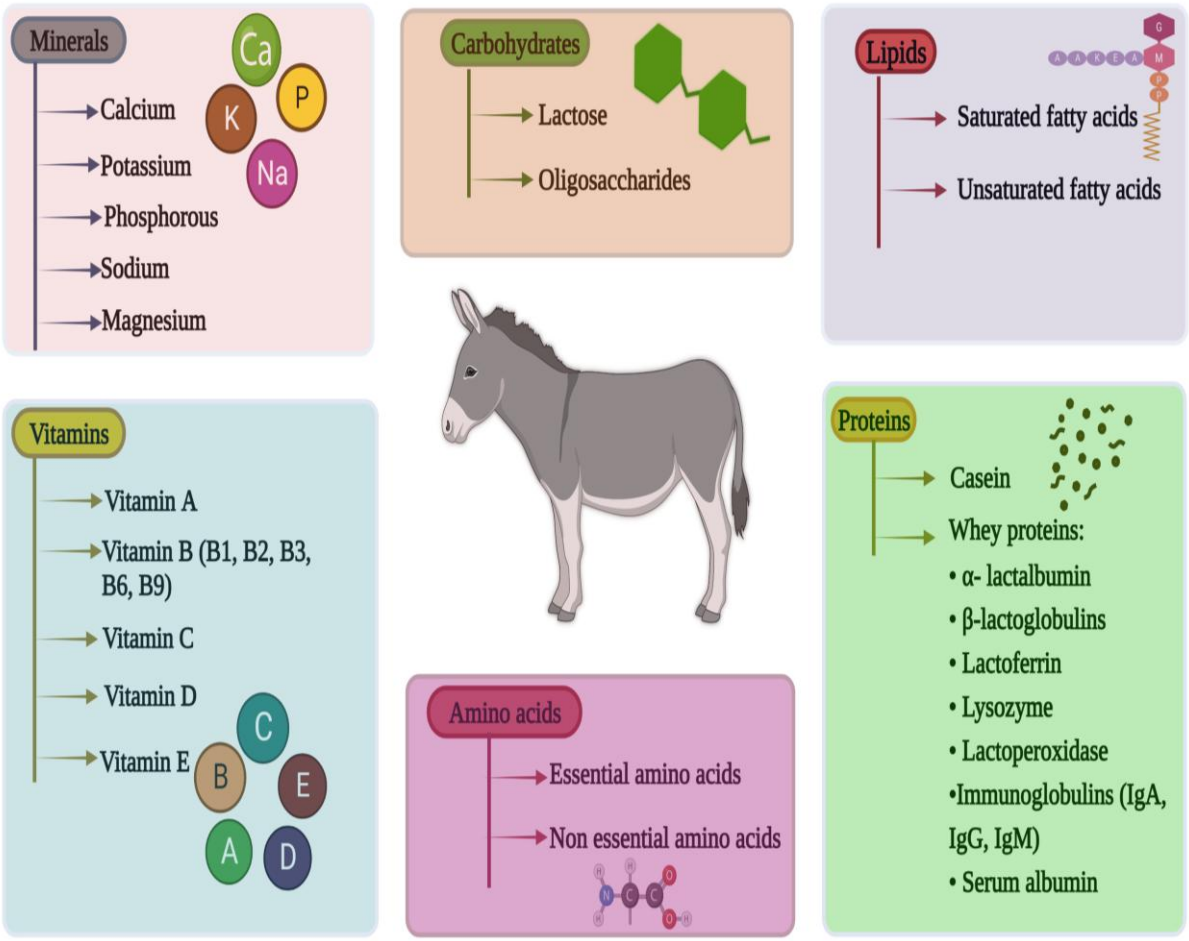

Figure 2 Physico-chemical constituents of donkey milk (Designed with Biorender premium software; https://app.biorender.com/)

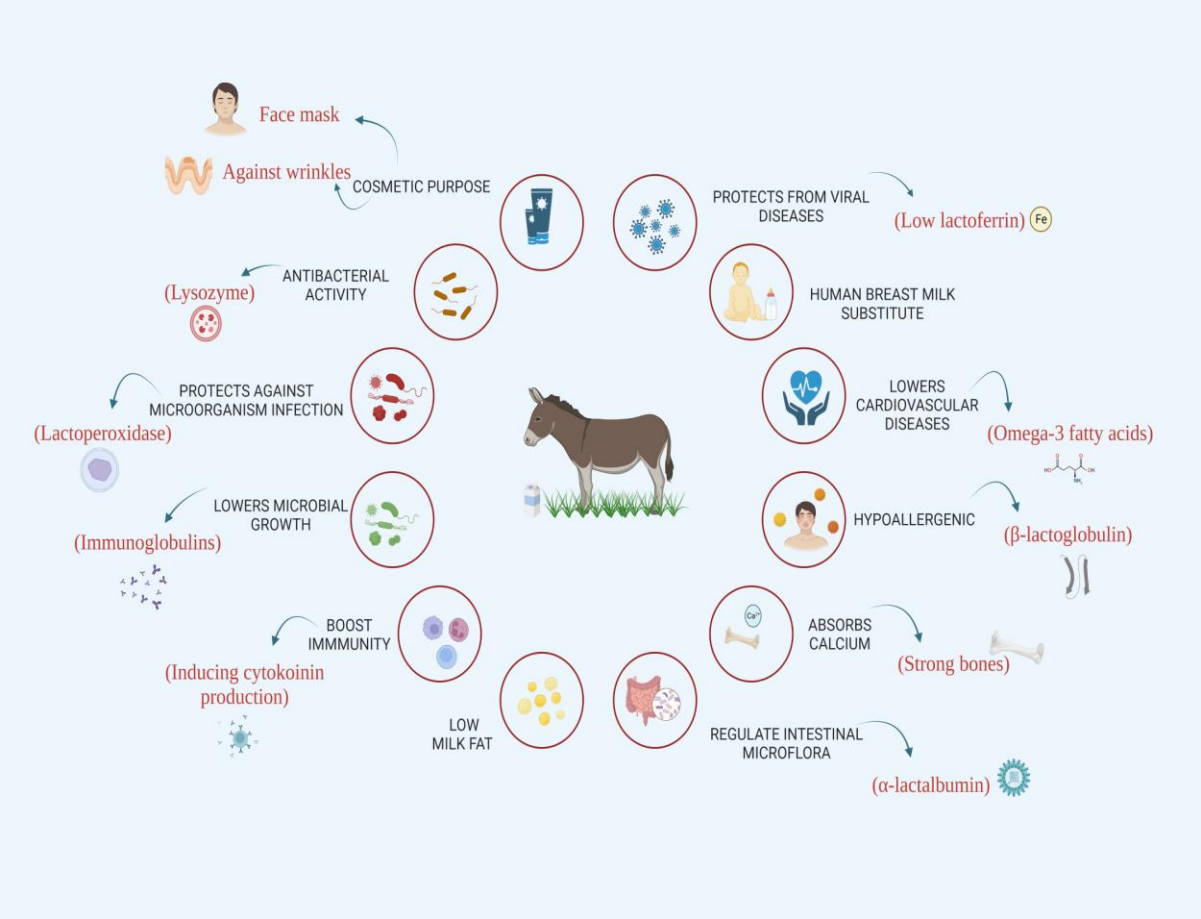

Figure 3 Substantial nutritional, therapeutic, and dietary implications of donkey milk for human beings
(Designed with Biorender premium software; https://app.biorender.com/)

has attracted much interest due to its ability to reduce allergens and strengthen the immune system. Donkey milk proteins are more evenly distributed between casein and whey than in bovine milk, which contains roughly five times more casein than whey (Garhwal et al. 2022). In contrast to cow's milk, donkey milk has much less protein casein, making it suitable for consumption by those sensitive to cow's milk (Martini et al. 2021; Papademas et al. 2022). Swapping from cow milk to donkey milk was safe for 81 Italian kids with cow milk allergies, and they continued to grow and gain weight usually. The sugar lactose is also vital to donkey milk (Mottola et al. 2018). Absorption of calcium, necessary for healthy bones, is facilitated. Moreover, donkey milk contains vitamin D, which may contribute to a stronger immune system and fewer cardiovascular issues (Bhardwaj et al. 2020; Cimmino et al. 2023). Laboratory tests showed that donkey milk stimulated the creation of cytokines, a protein that helps the body's immune system fight off infections. Donkey milk promotes nitric oxide production, which lowers blood pressure, relaxes blood vessel walls and boosts blood flow (Aspri et al. 2017; Papademas et al. 2022).

Lysozyme, the principal antibacterial agent in donkey milk, was reported in significant concentrations by both Tidona et al. (2015) and Nazzaro et al. (2010). Lactic acid bacteria (LAB) predominate in the microbiome of donkey milk. 80-90% of the total microbial load is typically LAB, with concentrations ranging from 3-4 log CFU/mL. Due to the antibacterial effect of lysozymes, the number of lactic acid bacteria (LAB) in donkey milk decreased during cold storage (Prasad 2020). One strain of *Lactococcus paracasei* that produces bacteriocin was found to have been isolated from donkey milk. *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli* are just a few of the harmful bacteria that were discovered to be vulnerable to the bacteriocin's antimicrobial effects (Cimmino et al. 2023). Similarly, Murua et al. (2013) found that donkey milk isolates of *Lactobacillus plantarum* produced a bacteriocin (LP08AD) that inhibited the development of food spoilage bacteria, LAB, and microorganisms (*Enterococcus faecium*, *Lactobacillus curvatus*, and *Listeria monocytogenes*). Additionally, it was discovered by Papademas et al. (2022) that raw donkey milk contained three different Enterococcus bacterial strains (type A, B, P) that produced enterocins, which are mainly effective against *L. monocytogenes*.

Three proteins with antibacterial effects could be measured in milk from a donkey, a human, and a cow. Lysozyme and lactoferrin are in high concentrations in human and donkey milk, but lactoperoxidase is present in trace levels (Garhwal et al. 2022). Donkey milk has a lysozyme concentration of 1 g/L, human milk of 0.12 g/L, and bovine milk of only trace concentrations, all lower than those found in donkey and human milk (Mottola et al. 2018). Donkey milk has less lactoperoxidase (0.11 mg/L) than human milk (0.77 mg/L) or bovine milk (30-100 mg/L). Donkey milk contains less lactoferrin (0.080 g/L) than human (0.3-4.2 g/L) or bovine (0.10 g/L) milk (Cimmino et al. 2023). Hydrolysis of glycosidic linkages of the mucous polysaccharides in the bacterial cell walls is the antimicrobial mechanism by which the iron-binding protein lactoferrin exerts its antibacterial effects (Chandran et al. 2020; Prasad 2020).

Food allergies to cow milk proteins are relatively frequent in infants and toddlers. Yet, donkey milk has no reported side effects which is utilized as natural hypoallergenic milk (Souroullas et al. 2018). Donkey milk has been shown to be less allergenic than cow milk in scientific research. Because of its high concentration of bioactive compounds, it was utilized to nourish European orphans and sick children until the end of the 20th century (Papademas et al. 2022). Regarding the lactose, protein, and minerals it contains, donkey milk is similar to human breast milk. Infants' immune systems were likely strengthened by the donkey milk's immunoactive compounds, including lysozyme and lactoferrin (Souroullas et al. 2018; Bhardwaj et al. 2020). Moreover, donkey milk has been found to have no negative impact on children's growth rate (Kumar et al. 2021). Milk from a donkey on a vegetable oil diet (4 mL/100 mL) is a good substitute for human breast milk (Madhusudan et al. 2017; Massouras et al. 2020).

Human-like leptin, ghrelin, triiodothyronine and insulin-like growth factor 1 are present in donkey milk, along with other growth factors and hormones. These molecules directly influence metabolism, body composition, and satiety regulation (Souroullas et al. 2018; Bhardwaj et al. 2020; Cimmino et al. 2023).

Preventing gastrointestinal infections in newborns may benefit from using lysozyme, lactoperoxidase, and lactoferrin, all of which have been shown to have antimicrobial and bacteriostatic properties (Chandran and Radhakrishnan 2019; Massouras et al. 2020). Because of their efforts, fresh donkey milk may be preserved for longer, which could increase its commercial viability. In addition to protecting against cancer development and metastasis, the iron-binding protein lactoferrin also plays a role in regulating iron homeostasis, cellular growth, fighting off infections, and inhibiting the spread of cancer (Ragona et al. 2016; Chandran et al. 2020; Martini et al. 2021). Compared to other milk proteins, it is very resistant to proteolytic degradation because it is from the transferrin family of proteins. It maintains a healthy gut microbiota by regulating the ratio of beneficial to harmful bacteria and encouraging the growth of Lactobacillus and Bifidobacterium (Mottola et al. 2018). Reverse phase high-performance liquid chromatography (RP-HPLC) research revealed that alpha-lactalbumin, lysozyme, and beta-lactoglobulin concentrations fluctuated during donkey lactation (60, 90, 120, 160, and 190 days postpartum) (Souroullas et al. 2018; Prasad 2020).

Perna et al. (2015) found that donkey milk fermented into a probiotic beverage is an effective way to introduce beneficial bacteria to the human digestive system. This probiotic drink can serve as a model for creating lactose-free, antioxidant-rich nutraceutical foods. As these microorganisms generate chemicals that alter the qualities of milk, the concentration of milk microbiota influences its chemical makeup. Donkey milk fermented with *Lactobacillus fermentum* ME-3 and *Lactobacillus acidophilus* (ATCC4356) was investigated for its probiotic, antioxidant, antibacterial, and sensory qualities by Papademas et al. (2022). Milk's high calcium and saturated fatty acid content and its beneficial protein profile have been shown to improve the makeup of the microbiota in the human stomach. Fermented milk appears to provide similar health benefits, and a fermented beverage of donkey milk can be made by co-culturing the probiotic species *Lactobacillus plantarum* and *Streptococcus thermophilus*. The selected species of *L. plantarum* increase the growth rate of bacteria to attain that supplied pH in a condensed amount of time, and they also promote the growth of *S. thermophilus* in that environment. In monocultures, one would not have anticipated such exciting growth activity (Kumari and Sharma 2021).

In addition to a high lysozymal content, donkey milk has been found to include many other beneficial bacteria, including *Lactobacillus paracasei*, *Lactococcus lactis*, and *Carnobacterium maltaromaticum*, all of which are well-adapted prospective probiotics. Leuconostoc, Enterococcus, and Streptococcus were shown to be less common than *L. paracasei* and *L. lactis* in a recent study (Kumari and Sharma 2021; Bertino et al. 2022). In-vitro studies have shown that certain probiotic strains are more effective at inhibiting microbial growth, preventing oxidative damage, and slowing cell proliferation. Raw donkey milk samples collected from farms had the highest concentrations of *L. paracasei* and *Enterococcus faecalis*, while samples collected from local fields had the highest concentrations of *L. lactis* and *Lysinibacillus sphaericus*. This study by Cosentino et al. (2022) examined donkey milk's essential components and isolated bacteria with potential probiotic properties against stress circumstances like excess bile, low pH, etc. Donkey milk probiotics present a fascinating field for further study and a novel source for highly effective probiotics in fermented food products. Donkey milk's potential probiotics are shown in Figure 4.

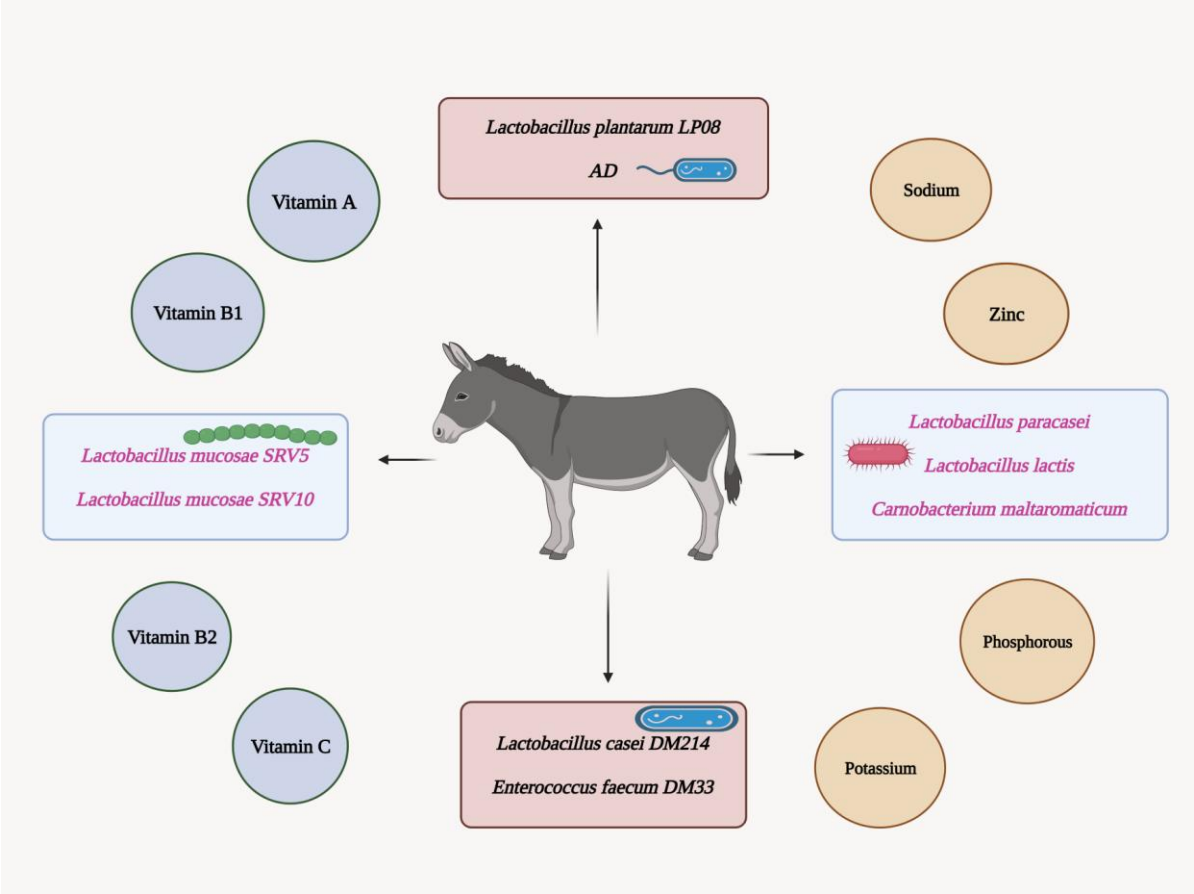

Figure 4 Potential probiotics in donkey milk (Designed with Biorender premium software; https://app.biorender.com/)

Donkey milk revealed a higher concentration of lysozyme than bovine milk (traces) or human milk (0.12 mg/mL), and this concentration increased significantly throughout lactation, with a mean value of 1.0 mg/mL (Bhardwaj et al. 2020). Donkey milk had a mean beta-lactoglobulin concentration of 3.75 mg/mL, similar to bovine milk (3.3 mg/mL). In the three months following delivery, the beta-lactalbumin concentration reached 1.8 mg/mL, very near to the beta-lactalbumin concentration in human milk (1.6 mg/mL) (Papademas et al. 2022). Donkey milk may have anti-inflammatory and antibacterial properties due to the presence of lysozyme. Other physiological roles for lysozyme include virus inactivation, immunoregulatory action, anti-inflammatory and antitumor effects (Ragona et al. 2016). Several investigations have shown that lysozyme has potent antitumor and angiogenic inhibitory properties. Donkey milk has a high concentration of lysozyme, which has antitumor properties. Alpha-lactalbumin, beta-lactoglobulin, blood serum albumins, immunoglobulins, lactoferrin, and lysozyme are the fundamental whey proteins found in donkey milk (Madhusudan et al. 2017; Massouras et al. 2020). Donkey milk has beta-lactoglobulin as a monomer, which is easier for infants to digest and absorb than it is in cow's milk. Strong anti-proliferative activity and potential utility in lung cancer treatment have been attributed to whey protein isolated from donkey milk; however, these claims require verification in vivo. The amount of whey protein in donkey milk also changes according to the time of lactation (Brumini et al. 2016; Souroullas et al. 2018; Bhardwaj et al. 2020). Better infant nutrition may be possible with a more thorough understanding of protein composition and variability. Donkey milk, especially collected in the first and second half of lactation when antimicrobial chemicals are at their highest, may positively affect digestive and immune system functioning (Aspri et al. 2017; Mottola et al. 2018).

Capillary gas chromatography can be utilized to ascertain the fatty acid profile of the milk sample. The PUFAs are crucial to the maturation of the newborn brain, the visual system, and the mind. The high concentration of omega-3 fatty acids in donkey milk may profoundly affect a baby's brain, eye, and body growth (Baloš et al. 2023). Omega-3 fatty acids reduce the risk of depression, cancer, dementia, and inflammatory illnesses such as dermatitis and rheumatoid arthritis (Madhusudan et al. 2017). Donkey milk's high PUFAs and omega-3 fatty acid concentration may aid the body's immune system. It has been shown that donkey milk can activate IgG secretion and release cytokines (like IL-1β, IL-10, IL-12 and

TNF-α), all of which are crucial in the immunological treatment of immune-related disease. Nitric oxide prevents atherosclerosis (Souroullas et al. 2018; Bhardwaj et al. 2020; Cimmino et al. 2023). Human peripheral blood mononuclear cells release nitric oxide after drinking donkey colostrum or milk. Donkey milk, however, can generate nitric oxide better than colostrum (Tafaro et al. 2007). Nitric oxide, at moderate intake levels (200 mL/day), can upregulate the immunological response in aged hosts; it is an efficient antibacterial agent in the formation of atherosclerosis; it is a potent vasodilator of terminal arteries; and it increases blood flow (Ragona et al. 2016). During lactation, the percentage of milk that contains unsaturated fatty acids (UFAs) rises to 48.022.97%. Over 180 days, the mean UFA/SFA ratio increased from 0.92 to 1.50, which is an extremely high value. The greater PUFA omega-3 to omega-6 ratio during month 7 of lactation is reflected in this finding (Garhwal et al. 2022).

Donkey milk has a better lipid pattern than cow milk, and its low amounts of docosahexaenoic acid and eicosapentaenoic acid may help treat atopy and cognitive development (Papademas et al., 2022). Because their fatty acid structure, especially that of long-chain fatty acids (LC-SFA), is identical to human milk, donkey milk lipids are easily absorbed despite their low content (Madhusudan et al. 2017). As part of a balanced diet, donkey milk provides essential fatty acids. Subjects with cow milk protein allergy, particularly those with numerous food allergies, have a heightened need for these fatty acids (Conte and Panebianco 2019).

Lactobacillus bacteria are commonly cultured in equine (horse and donkey) milk, making it a popular ingredient in probiotic beverages. Koumiss is a traditional beverage of central Asia made from fermented equine milk. Mongolia's national beverage is koumiss. In Mongolia, it is said that koumiss may treat 40 different illnesses (Aspri et al. 2017).

Lactobacillus rhamnosus is a beneficial bacterium that occurs naturally in the human microbiome and helps maintain a healthy gut environment and modulate the immune system on both the local and systemic levels (Brumini et al. 2016). Peng et al. (2014) examined the cellular, humoral, and nonspecific immune responses elicited by donkey milk. They found that it stimulated the production of interleukins by lymphocytes and macrophages, as well as the expression of activation cells surface molecules like CD25, which is used to monitor disease progression, and CD69, a signal-transmitting receptor in lymphocytes. These cytokines strengthen the immune system in donkey milk. Furthermore, nitric oxide is released from peripheral blood mononuclear cells after drinking donkey milk. Donkey milk's immunological properties—including its ability to serve as a vasodilator and a potent agent against infections and their products—may make it a beneficial tool in the fight against atherosclerosis (Derdak et al. 2020; Baloš et al. 2023). Donkey milk whey protein and whey hydrolysate have been studied for their potential impact on the physiological activity and gut microbiota of D-galactose-induced aged mice. This has been done to discover more effective anti-aging methods. Whey protein and whey hydrolysate have been proven to ameliorate the effects of aging on the body by decreasing levels of reactive oxygen species (ROS) and malondialdehyde (Zhou et al. 2022).

Humans have been drinking and applying donkey milk since prehistoric times, but it wasn't until the Renaissance that the practice was studied scientifically. Since then, it has been successfully utilized in Western Europe as a human milk substitute, a diet for infants with food allergies, to enhance the immunological response of otherwise healthy old individuals, and to manufacture fermented beverages (Souroullas et al. 2018; Bhardwaj et al. 2020). Moreover, anti-proliferative and anticancer activity was observed in vitro for whey proteins isolated from donkey milk. Because of its beneficial qualities, milk has widespread use in food and cosmetics (Brumini et al. 2016; Conte and Panebianco 2019). Donkey milk is nutritionally equivalent to human milk, could replace human breast milk and be used in alternative diets. In light of this, it has been proposed that infants and newborns who cannot get their mother's milk may benefit from consuming a milk powder made from donkey milk (Souroullas et al. 2018; Bhardwaj et al. 2020). Several scientific researches have demonstrated the skin-benefiting properties of cosmetics using donkey milk. Donkey milk's abundance of beneficial nutrients makes it an excellent choice for naturally nourishing and toning the skin. Donkey milk's natural antibacterial compounds, like lysozyme and lactoferrin, help prevent skin infections by keeping harmful microorganisms from multiplying (Massouras et al. 2020). These properties of chemically-enhanced donkey milk make it a promising treatment option for a wide range of skin disorders. Soap and face cream are not the only products that benefit from donkey milk today (Martini et al. 2021).

5 Novel food development applications of donkey milk

Donkey milk's capacity to include probiotic microorganisms for antibiotic-associated diarrhea prevention and therapy is an extra benefit. However, lactose sensitivity sufferers may not be able to drink this milk. Donkey milk is fermented with LAB that hydrolyzes lactose, casein, and whey protein, releasing a wide range of organic acids, peptides, and amino acids, allowing for the creation of novel foods for people who are intolerant to lactose or cow milk protein (Cimmino et al. 2023). Panellists thought donkey milk was delicious, describing it as white, thin, smelling sweet, tasting sweet, and leaving no aftertaste (Malissiova et al., 2016). When milk is used for manufacturing, the generated products should be examined for technological factors and food processing appropriateness. Donkey milk yogurt, probiotic yogurt, and fermented milk were rarely made (Perna et al., 2015). These products' sensory quality was consumer-acceptable, and LAB starter and probiotic cultures > 6 log CFUs/mL

survived storage, confirming their functional and probiotic qualities (Baloš et al. 2023).

Since donkey milk is in short supply, preserving it to keep it fresh for longer is crucial. Donkey milk can be bought in various forms, including raw, pasteurized, and ultra-high-temperature (UHT) versions. Other ways of donkey milk storage include freezing, freeze-drying (lyophilizing), and powdering (Papademas et al. 2022; Baloš et al. 2023). Donkey milk's basic components are stable through heating and storing. The nutritional value of fat and the overall composition of the food is unaffected by further freezing or refrigeration (Martini et al. 2018b).

Donkey milk was used by Tidona et al. (2015) to create a drink that was fortified with sunflower oil. Donkey milk naturally has low-fat content; however, adding sunflower oil to an emulsion raised various lipid-quality indicators and made the milk more viscous and stretchier. Pure camel chymosin, which clogs donkey milk casein micelles, was utilized to make soft cheese from raw donkey milk. Due to its reduced casein and total solids concentration, donkey milk yields extremely little cheese (Cimmino et al. 2023). Instead of egg lysozyme, donkey milk can reduce "blowing" in semihard and hard cheeses. Adding donkey milk to cow milk (2% to 8% v/v) increased lysozyme levels, and late-blowing defects in cheese decreased (Cosentino et al., 2015; Cosentino et al. 2016; Baloš et al. 2023). This was achieved without negatively impacting the sensory appeal of the cheese to customers.

Equine milk is typically produced on a small scale because of diversity in breed, feeding regime, etc., whereas cow milk is homogeneous in terms of fat and protein content due to its industrial processing on a large scale. A small but significant spread of such unique devices, each with the productivity of a jenny but with the potential to generate niche markets, is expected to join the market shortly. Donkey milk's appealing nutritional qualities could be used in various contexts (such as with the elderly population) or new food formulations, not just in baby diet treatment (Garhwal et al. 2022; Papademas et al. 2022; Baloš et al. 2023; Cimmino et al. 2023).

Conclusion and future prospects

Donkeys have been a part of human history. The mules help with farming and other outdoor tasks. Nonetheless, female donkeys are highly prized for their milk. The health benefits of donkey milk have been claimed to be similar to those of human breast milk. In the late 20th century, orphans and unwell children throughout Europe were fed donkey milk. Donkey milk is also helpful for people with cow milk protein allergies because it causes fewer allergic reactions. The mild sweetness makes this a good candidate for the kid's menu. Vitamin C (60 times more than cow's milk), E, D, E, omega-3 and omega-6 fatty acids, zinc, magnesium and calcium may all be found in plenty in donkey milk, making it an excellent choice for a child's diet. Donkey milk is effective against various respiratory illnesses, including bronchitis, tuberculosis, cough, pneumonia, and asthma. It is also beneficial for patients with bone problems like osteoporosis due to the high levels of calcium and vitamin D. In the cosmetics industry; it is used in a wide range of soap and cream formulations. Donkey milk's physiological and nutritional advantages need more research and innovation.

Donkey milk contains immunoglobulins, vitamins, minerals and omega fatty acids. It's a good alternative for infants not breastfed because it's quickly absorbed. Children with an allergy to the protein found in cow's milk can also benefit from this camel milk. There are numerous aesthetic and therapeutic uses for donkey milk. It is a key ingredient in making dairy goods like ice cream, cheese, milk powder, yoghurt, and more. Further research into the healing and nutritional benefits of donkey milk is encouraged. Also, efforts should be made to lower the price of donkey milk so that it is affordable even for those on tight budgets. We must know that donkey milk can be part of a healthy diet.

Donkey milk resembles human milk more than ruminant milk. Protein, lactose, linolenic acid, vitamin C and mineral levels match breast milk. The protein part is more easily absorbed than proteins in bovine milk, and the total milk lipid content is lower than that of human and bovine milk. Current research has demonstrated its usefulness as a vitamin D source. Donkey milk is suitable for kids allergic to bovine milk proteins. As a result, it is suggested that this milk can be used in paediatrics, and it has the potential to serve as a superior substrate to cow's milk in the production of infant formulae. Adequate fat increases and alterations in certain fatty acid levels are necessary for this formulation. Because of its vitamin D concentration and its anti-inflammatory, anticancer, and hypolipidemic activities (all of which have been demonstrated in experimental models), it may help treat obesity and the care of the aged. Donkey milk would be yet another alternative food with consumers' health in mind. New well-designed clinical studies in vulnerable populations are needed to validate and update the putative health benefits.

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